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Flavonoids of *Guiera senegalensis* J. F. GMEL. – Thin-layer Chromatography and Numerical Methods*

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The efficiency of seven thin-layer chromatographic systems for separating nine flavonoids isolated from *Guiera senegalensis* J. F. GMEL. (*Combretaceae*) was investigated. For this purpose, three mathematical techniques were applied. The first technique was the calculation of the information content derived from Shannon's equation for each chromatographic system. In the second technique, the discriminating power of the systems was measured individually and in various combinations. The third technique classifies chromatographic systems according to clusters. The classification was carried out by the numerical taxonomy methods. The most suitable chromatographic systems for the separation of the investigated flavonoids are: ethyl acetate : ethylmethylketon : formic acid : water, 60:15:3:2, and ethyl acetate : formic acid : acetic acid : water, 100:11:11:27.

INTRODUCTION

Guiera senegalensis J.F.GMEL. (*Combretaceae*) is a shrub of the savannah region of West and Central Africa. Its leaves, 3-5 cm long and 1.5-3 cm broad, are opposite or subopposite, oblong-elliptic, rounded or slightly cor-

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date at base, mucronate at apex. They are softly tomentose on both surfaces, with scattered black glands beneath.[1]

Guiera senegalensis is widely used in the traditional medicine of western Africa.[2–5] In Ghana, e.g., leaves are used against dysentery, diarrhoea, gastro-intestinal pains and disorders, rheumatism and fever.[3] Previous investigations into the prostaglandin biosynthesis showed a small inhibitory activity.[6] Mucilagines, tannins, flavonoids, alkaloids and amino acids are the so far known constituents of *Guiera senegalensis*.[2,4,7] From the methanolic extract of the dried leaves of this plant, flavonol aglycones as well as flavonol glycosides, some of them acylated, were isolated.[8]

Thin-layer chromatography (TLC) is an ideal technique for the screening of drugs because of its low cost, easy maintenance and selectivity of detection reagents. TLC on silica gel is very favourable for the analysis of flavonoids.[9,10]

In this paper, the efficiency of seven thin-layer chromatographic systems for the separation of nine flavonoids isolated from the leaves of *Guiera senegalensis* was investigated by the methods of numerical taxonomy. The effectiveness of chromatographic systems is measured in terms of the selectivity and probability of separating two flavonoids, randomly selected from a specific population.[11] The measure of selectivity is the information content, and the discriminating power is the measure of probability.[12–14] The methods of numerical taxonomy classify the chromatographic systems according to clusters.[15,16] The chromatographic systems are divided into groups with similar separation properties. The selection of the most efficient chromatographic system from each group is carried out according to the information content or to the discriminating power.[15]

In this paper we have used known numerical methods, with our original computer search program.[12,16] The application of these methods in the investigation of flavonoids is original.

EXPERIMENTAL

Materials

TLC experiments were carried out with methanolic solutions (0.5 mg/mL) of the isolated flavonoids. The seven TLC systems used are given in Table I.[17–20]

All TLC separations were performed with silica gel plates incorporating a fluorescent indicator (Kieselgel 60 F_{254}-Alufolien, 20×20 cm, 0.25 mm thickness, Art. No. 1.05554, Merck, Darmstadt, Germany). TLC tanks were allowed to equilibrate for at least 60 min. 5 μL of flavonoid solutions were applied to the plates and the systems were allowed to run for 15 cm. Visualization of the flavonoids was attained by spraying the sheets with 1% methanolic diphenylboryloxyethylamine, followed by 5% ethanolic polyethylene glycole 4000. The chromatograms were evaluated in UV light

TABLE I

The thin-layer chromatographic systems studied

System No.	Solvent	Time of development (min)	Ref.
1	Ethyl acetate:ethylmethylketon: formic acid:water (50:30:10:10)	25	17
2	Ethyl acetate:formic acid:water (68:8:8)	30	18
3	Ethyl acetate:ethylmethylketon:formic acid:water (60:15:3:2)	20	*
4	Ethyl acetate:methanol:water (100:13.5:10)	25	19
5	Ether:dioxan:ethyl acetate:formic acid:water (50:15:30:3:2)	26	20
6	Ethyl acetate:methanol:formic acid:water (100:13.5:2.5:10)	25	*
7	Ethyl acetate:formic acid:acetic acid:water (100:11:11:27)	40	19

* own modifications

(366 nm).[19] The structures of the flavonoids isolated from *Guiera senegalensis* and analyzed by TLC are presented in Table II.

Methods

Calculation of the Information Content

The generation of information can be considered as the reduction of uncertainty with respect to the composition or identity of the sample to be analyzed. This implies that any uncertainty remaining after analysis can be treated as a parameter for the evaluation of analytical results.[21]

Extensive information has been calculated for seven TLC systems by Shannon's formula. Distribution of R_F values of the isolated flavonoids into groups with error factor E (*e.g.* $E = 0.05$ or $E = 0.10$) with respect to R_F units and the assumption of n_k R_F values in the k-th groups, the entropy (average information content) is given by the following Shannon equation.[21–23]

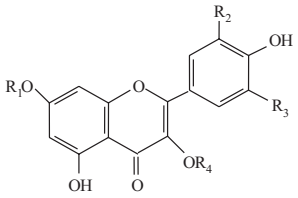
$$I(X) = H(X) = -\sum_k \frac{n_k}{n} ld \frac{n_k}{n} [bit] \quad (1)$$

where *I(X)* is an average information content and *H(X)* is the entropy.[12]

It is also assumed that the flavonoids with R_F values within one group cannot be identified. It is obvious that entropy is at its highest level if there is only one R_F value, *i.e.* $H_m(X) = ld\ n$ within each group.

TABLE II

Structures of the isolated flavonoids



Flavonoid No.	Name of the flavonoid	R_1	R_2	R_3	R_4
1	Myricitrin	H	OH	OH	α-L-rha p
2	Myricetin-3-*O*-β-D-glucopyranoside	H	OH	OH	β-D-glu p
3	Myricetin-3-*O*-β-D-galactopyranoside	H	OH	OH	β-D-gal p
4	Myricetin-3-*O*-β-D-(6''-*O*-galloyl)-glucopyranoside	H	OH	OH	6''-G-β-D-glu p
5	Myricetin-3-*O*-α-L-arabinopyranoside	H	OH	OH	α-L-ara p
6	Quercitrin	H	OH	H	α-L-rha p
7	Quercetin-3-*O*-α-L-arabinopyranoside	H	OH	H	α-L-ara p
8	Rhamnetin	CH_3	OH	H	H
9	Tiliroside	H	H	H	6''-TPC-β-D-glu p

rha p = rhamnopyranoside; glu p = glucopyranoside; gal p = galactopyranoside; G = galloyl; ara p = arabinopyranoside; TPC = *trans*-p-coumaryl

Determination of Discriminating Power

Discriminating power (*DP*) is used as a measure of the effectiveness of chromatographic systems. The *DP* of a chromatographic system is the probability of separating two flavonoids selected at random from a specific substance population.[14] Two flavonoids are chromatographically similar if the differences in their identification values do not exceed the error factor *E*.

The *DP* of a set of chromatographic systems is defined as the probability of identifying two randomly selected flavonoids in at least one of the systems.[24–28] It must be possible to discriminate all pairs of *N* in order to compute the *DP* of *k* chromatographic systems in which *N* flavonoids are investigated. For the total number of matching pairs (*M*), the probability of a random selection of chromatographically similar pairs is $2M/N(N-1)$. Therefore, the *DP* of *k* systems is:

$$DP_k = 1 - \frac{2M}{N(N-1)} \,. \tag{2}$$

Calculation of the DP values and their maximization can be more easily attained by providing the following conditions: an even distribution of R_F values, reproducibility of results and no correlations between chromatographic systems.[26,28]

The average number of chromatographically similar flavonoids (T) for the chromatographic systems considered can be calculated from the following equation:

$$T = 1 + (N-1)(1 - DP_k) \,. \tag{3}$$

Calculation of Taxonomic Distances, Cluster Formation and Dendrogram

Taxonomy is defined as the theoretical study of classification, including its elementary principles, procedures and rules.[15] Numerical taxonomy deals with the ways of classifying chromatographic systems into taxonomic groups based on the R_F values. The mathematical principle of this procedure is based on the formation of a matrix with columns representing the solvent systems and rows the flavonoids. Classification is carried out with respect to resemblances between the solvent systems. The optimal combination of two or more chromatographic systems for the separation of flavonoids by TLC can be readily determined from taxonomic distances.[29]

Taxonomic distance is inversely related to similarity. The greater the differences in the properties of solvent systems, the larger are their spatial distances. The distance $d_{j,k}$ between solvent systems j and k is equal to:

$$d_{j,k} = \sqrt{\sum_{i=1}^{N} (X_{i,j} - X_{i,k})^2 / N} \tag{4}$$

where $X_{i,j}$ *and* $X_{i,k}$ are the R_F values of the investigated flavonoid i in the solvent systems j and k and N is the number of flavonoids taken into account.

Chromatographic systems with a high degree of resemblance are grouped into clusters. Cluster formation in this paper was carried out by a weighted pair group method using the arithmetic average.[15] The smallest distance $d_{j,k}$ or the highest correlation coefficient (r>0.95) between solvents j and k is selected: j and k are the most similar solvent systems and are therefore considered to form one group p'. The similarity coefficient between the new group p' and all the other phases *(e.g.* q) is calculated, *e.g.* for the distance, as follows:

$$d_{j,p'} = \frac{1}{2}\left(d_{j,p'} + d_{j,q}\right) \,. \tag{5}$$

The total number of rows and columns in the resemblance matrix is, therefore, reduced to one. This process is repeated until all chromatographic systems are comprised in one non-overlapping hierarchic system of groups and subgroups (clusters). The procedure for cluster formation is presented by a dendrogram.[30–33] The three approaches were compared applying our computer search program KT 1.[12]

The optimal combination of two or more systems was selected using the following procedures:

a) determination and comparison of the amount of information and discriminating power for all possible combinations of chromatographic systems,

b) classification of chromatographic systems into groups with similar separation properties and selection of the most efficient chromatographic system from each group

RESULTS AND DISCUSSION

A data set of R_F values for the separation of flavonoids isolated from *Guiera senegalensis* (Table II) into seven different chromatographic systems (Table I) was analyzed.

Table III gives input data for the investigated flavonoids. Table IV gives output data for the discriminating power and the information content for each TLC system and Table V gives output data for combined systems $K = 2$ and $K = 3$; K is the number of combined systems.

Under the conditions most frequently used in chromatographic analysis, *i.e.* $E = 0.05$, the most suitable systems for separating the flavonoids studied were the chromatographic systems **1** (ethyl acetate: ethylmethylketon:formic acid:water, 50:30:10:10) and **3** (ethyl acetate:ethylmethylketon:formic acid:water, 60:15:3:2) because they showed the largest information content

TABLE III

Input data: R_F values of the flavonoids isolated from *Guiera senegalensis*

Flavonoid	Solvent system*						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1. Myricitrin	0.70	0.57	0.33	0.44	0.15	0.50	0.62
2. Myricetin-3-*O*-β-D-glucopyranoside	0.56	0.42	0.18	0.41	0.11	0.40	0.50
3. Myricetin-3-*O*-β-D-galactopyranoside	0.52	0.39	0.15	0.41	0.09	0.40	0.46
4. Myricetin-3-*O*-β-D-(6''-*O*-galloyl)-glucopyranoside	0.60	0.46	0.25	0.37	0.14	0.49	0.52
5. Myricetin-3-*O*-α-L-arabinopyranoside	0.63	0.50	0.26	0.44	0.17	0.47	0.55
6. Quercitrin	0.80	0.66	0.44	0.51	0.29	0.59	0.71
7. Quercetin-3-*O*-α-L-arabinopyranoside	0.72	0.57	0.26	0.49	0.24	0.54	0.62
8. Rhamnetin	0.98	0.95	0.86	0.00	0.88	0.87	0.96
9. Tiliroside	0.90	0.82	0.58	0.62	0.40	0.72	0.85

* Copies of chromatograms can be obtained from the authors on request

TABLE IV

Output data for *DP* and *I* in the range of error factors for each chromatographic system

TLC-system	E = 0.05		E = 0.10	
	DP	*I* (bit)	*DP*	*I* (bit)
1	0.8889	2.948	0.6667	2.197
2	0.8889	2.725	0.7222	2.419
3	0.8889	2.948	0.6944	2.419
4	0.7500	2.281	0.4722	1.880
5	0.7778	2.642	0.6111	2.059
6	0.8333	2.419	0.5556	2.197
7	0.8889	2.503	0.6944	2.503

TABLE V

Output data for *DP* and *T* for combined solvent systems K = 2 and K = 3

	Combination sequence	Solvents	E = 0.05	
			DP	T
K = 2	1.	**3–4**	0.9722	1.222
	2.	**4–7**	0.9444	1.444
	3.	**3–7**	0.9444	1.444
	4.	**3–6**	0.9444	1.444
	5.	**3–5**	0.9444	1.444
	6.	**2–4**	0.9444	1.444
	7.	**2–3**	0.9444	1.444
	8.	**1–4**	0.9444	1.444
	9.	**1–3**	0.9444	1.444
	10.	**6–7**	0.9167	1.667
K = 3	1.	**4–6–7**	0.9722	1.222
	2.	**3–4–7**	0.9722	1.222
	3.	**3–4–6**	0.9722	1.222
	4.	**3–4–5**	0.9722	1.222
	5.	**2–4–6**	0.9722	1.222
	6.	**2–3–4**	0.9722	1.222
	7.	**1–4–6**	0.9722	1.222
	8.	**1–3–4**	0.9722	1.222
	9.	**5–6–7**	0.9444	1.444
	10.	**4–5–7**	0.9444	1.444

(I = 2.948) and a high discriminating power (DP = 0.8889). Systems **2** (ethyl acetate:formic acid:water, 68:8:8) and **7** (ethyl acetate:formic acid:acetic acid:water, 100:11:11:27) are also suitable because of their identical DP value (DP = 0.8889) to systems **1** and **3**. The results made it evident that at E = 0.10 the most appropriate systems for separating the flavonoids studied are systems **2** (DP = 0.7222, I = 2.419), **3** (DP = 0.6944, I = 2.419) and **7** (DP = 0.6944, I = 2.503).

Combining two chromatographic systems with the error factor E = 0.05 all the systems show high DP values (DP = 0.9722, 0.9444 and 0.9167). The number of flavonoids with similar chromatographic properties (T) is 1.222, 1.444 and 1.667. Systems **3** and **7** can often be found in the first ten combinations. In a series of three systems at the same error factor, systems **3** and **7** came in the first four combinations (DP = 0.9722, T = 1.222).

The same results were obtained by cluster formation (Table VI) and from dendrogram (Figure 1). In order to obtain the optimal combination of two chromatographic systems, according to the dendrogram (Figure 1), system **3** should be chosen from cluster 3 and system **7** from cluster 1.

TABLE VI

Formation of clusters

Cluster	Solvent	Solvent	Distance
1	2	7	0.0536
2	2	6	0.0760
3	3	5	0.1139
4	1	2	0.1307
5	1	2	0.3419
6	1	2	0.3561

CONCLUSIONS

Thin-layer chromatography is a procedure in which different chemometrical methods are used most frequently. The combined use of mathematical tools, such as information theory and numerical taxonomy, permits the classification and combination of chromatographic techniques. They should therefore be of value in comparative physicochemical studies of these systems and in the selection of sets of preferred solvent systems.

In this paper, the separating power of different TLC systems described in literature and own modifications of them are compared by numerical taxonomy methods. The proposed calculations point to the conclusion that for the TLC analysis of flavonoids isolated from *Guiera senegalensis* J. M. GMEL.,

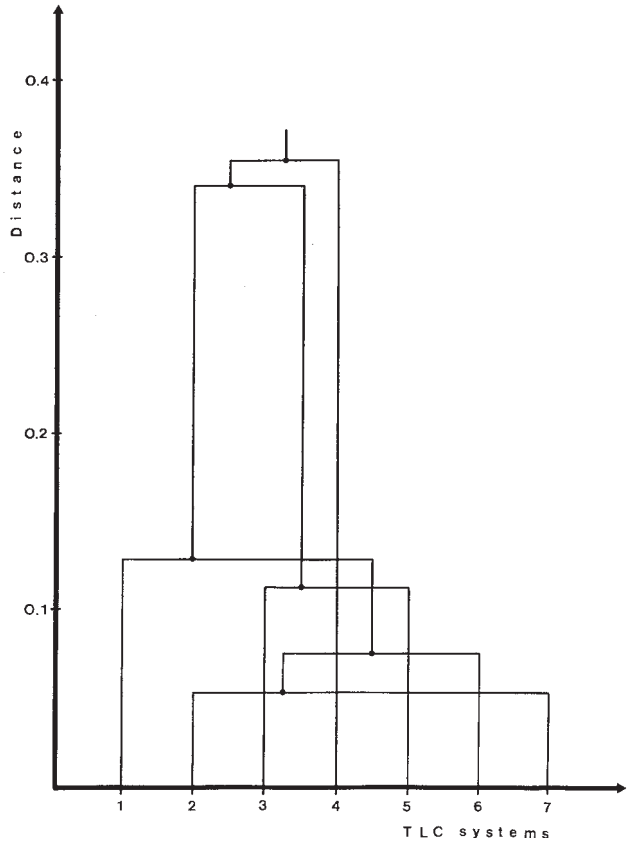


Figure 1. Dendrogram for seven TLC systems.

chromatographic systems ethyl acetate:ethylmethylketon:formic acid:water, 60:15:3:2, and ethyl acetate:formic acid:acetic acid:water, 100:11:11:27 are the most suitable. The suggested solvent combinations proved an exellent reproducibility of the results and an even distribution of the R_F values.

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SAŽETAK

Flavonoidi biljne vrste *Guiera senegalensis* J.F. GMEL. – tankoslojna kromatografija i numerički postupci

Željan Maleš, Marica Medić-Šarić i Franz Bucar

Istražena je uspješnost sedam razvijača uporabljenih u tankoslojnoj kromatografiji za odjeljivanje devet flavonoida, koji su izolirani iz biljne vrste *Guiera senegalensis* J.F. GMEL. (*Combretaceaeae*). U tu svrhu uporabljena su tri matematička postupka. Prvi postupak temelji se na stvaranju svih mogućih kombinacija traženog broja kromatografskih razvijača, te određivanju srednjeg vlastitog sadržaja informacije izvedenog iz Shannon-ove jednadžbe. Drugi postupak obuhvaća vjerojatnost da se dva flavonoida izabrana slučajno iz promatrane skupine flavonoida mogu razlikovati tim razvijačem, a izražava se koeficijentom *DP* (engl. Discriminating Power). U okviru trećeg postupka kromatografski razvijači svrstavaju se u skupine-grozdove različitih identifikacijskih karakteristika. Svrstavanje razvijača temelji se na njihovoj međusobnoj sličnosti, a provodi se postupcima numeričke taksonomije. Najprikladniji razvijači za odjeljivanje istraživanih flavonoida su: etilacetat:etilmetilketon:mravlja kiselina:voda, 60:15:3:2 i etilacetat:mravlja kiselina:octena kiselina:voda, 100:11:11:27.